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(54) FAT EMULSIONS FOR INHALATIONAL ADMINISTRATION

(57) The object of the present invention is to provide a pharmaceutical composition optimized for the administration of a drug, particularly a drug which is only sparingly soluble in water, by way of inhalation.

The present invention is a fat emulsion for inhalational administration, or a lyophilized composition thereof, which is an o/w fat emulsion comprising fat emulsion particles essentially composed of an oil component, an emulsifying agent and a drug as dispersed in water, characterized in that the average particle diameter of said fat emulsion particles lies within the range of 5~100 nm.

With the aid of a suitable inhaler, the inhalant of the invention readily yields a mist of aerosol particles fine enough to reach the alveolus; the inhalant is well amenable to size control of the aerosol particles.

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4000~6000), polyoxyalkylene copolymers (e.g. a polyoxyethylene-polyoxypropylene copolymer with an average molecular weight of 1000~20000, preferably 6000~10000), hydrogenated castor oil polyoxyalkylene derivatives (e.g. hydrogenated castor oil polyoxyethylene(20) ether, do(40) ether, do(100) ether, etc.), and castor oil polyoxyalkylene derivatives (e.g. castor oil polyoxyethylene(20) ether, do(40) ether, do(100) ether, etc). These can be used each alone or in a combination of two or more species. The preferred emulsifying agent includes egg yolk phosphatidylcholine, egg yolk lecithin and soybean lecithin, among others. For practical purposes, egg yolk lecithin and soybean lecithin are preferred.

[0012] The level of said emulsifier in the inhalant of the invention should vary with the species of emulsifier and other components but may appropriately be 0.05~40 w/v %, preferably 0.1~20 w/v %.

[0013] The oil component-to-emulsifying agent (oil/emulsifier) ratio by weight may be 0.1~20, preferably 0.4~6.0, more preferably 0.8~1.2 (particularly 1).

[0014] The drug which can be used in the present invention is not particularly restricted but is preferably a drug which is more readily lipid-soluble than water-soluble. As such drugs, the so-called lipid-soluble drugs and water-insoluble drugs can be mentioned. Included among them are central nervous system drugs, peripheral nervous system drugs, sensory organ drugs, cardiovascular system drugs, respiratory system drugs, hormones, urogenital system drugs, drugs for anal diseases, vitamins, drugs for liver diseases, antigout drugs, enzymes, antidiabetics, immunosuppressants, cytoactivators, antitumoral drugs, radioactive drugs, antiallergic drugs, antibiotics, chemotherapeutic agents, biological drugs, and extracorporeal diagnostic agents.

[0015] More particularly, the following drugs can be mentioned by way of example.

1. Steroidal drugs

[0016] Dexamethasone, prednisolone, betamethasone, beclomethasone propionate, triamcinolone, hydrocortisone, fludrocortisone and prasterone, salts thereof, and their lipid-soluble derivatives.

2. β-Adrenergic agonists

[0017] Procaterol, orciprenaline, isoproterenol hydrochloride, pirbuterol, terbutaline, hexoprenaline, fenoterol hydrobromide, hexoprenaline sulfate, terbutaline sulfate, salbutamol sulfate, oxyprenaline sulfate, formoterol fumarate, isoprenaline hydrochloride, pirbuterol hydrochloride, procaterol hydrochloride, mabuterol hydrochloride, and tulobuterol, salts thereof, and their lipid-soluble derivatives.

3. Xanthine derivatives

[0018] Diprophylline, proxyphylline, aminophylline and theophylline, salts thereof, and their lipid-soluble derivatives.

4. Antibiotics

[0019] Pentamidine isethionate, cefmenoxime, kanamycin, fradiomycin, erythromycin, josamycin, tetracycline, minocycline, chloramphenicol, streptomycin, midecamycin, amphotericin B, itraconazole and nystatin, salts thereof, and their lipid-soluble derivatives.

5. Others

[0020] Ipratropium bromide, methylephedrine hydrochloride, trimethoquinol hydrochloride, clenbuterol hydrochloride, oxitropium bromide, fultropium bromide, methoxyphenamine hydrochloride, chlorprenaline hydrochloride sodium cromoglycate.

[0021] The formulating level of the drug in the inhalant of the invention varies with the species of drug and other components but may suitably be 0.05~20 w/v %.

[0022] Furthermore, in the present invention, a co-emulsifier and/or an emulsion stabilizer can be formulated. The co-emulsifier and/or emulsion stabilizer includes straight-chain or branched-chain saturated or unsaturated fatty acids containing 6~22 carbon atoms, such as stearic acid, oleic acid, linoleic acid, palmitic acid, linolenic acid, myristic acid, etc. and salts thereof [e.g. alkali metal salts (sodium salts, potassium salts, etc.), alkaline earth metal salts (calcium salts etc.)]; primary or secondary aliphatic amines containing 2~22 carbon atoms, such as ethanolamine, propylamine, octylamine, stearylamine, oleylamine, etc.; basic amino acids such as lysine, histidine, ornithine, arginine, etc.; sterole such as cholesterol, cholestanol, etc.; and charged substances such as phosphatidic acid, ganglioside, stearylamine, etc. These may be used each alone or in a suitable combination of two or more species.

[0023] The formulating level of these substances depends on the objective to be achieved but may generally be not

BEST MODE FOR CARRYING OUT THE INVENTION

[0036] The following examples and test examples are intended to illustrate the present invention in further detail.

Example 1

[0037] To 5 mg of cholesterylanthracene-9-carboxylate (CA), a fluorescent cholesterol derivative, as a mock drug, were added 500 mg of purified egg yolk lecithin, 500 mg of purified soybean oil, 9 mL of distilled water for injection and, further, 220 mg of glycerin J.P. and the mixture was sonicated with a probe type ultrasonic homogenizer (Branson Sonifier Model 185; the same applies hereinafter) under ice-water cooling for 60 minutes. The CA-containing fat emulsion thus obtained was light yellow and transparent. After the emulsion was diluted with distilled water for injection to make 10 mL, it was filtered through a 0.22 μm membrane filter to give a sterilized preparation, which was then filled in injection ampules, 2.0 mL/ampule, under nitrogen gas in a clean bench to prepare the inhalant of the invention. The average particle diameter of this inhalant fat emulsion as measured with a light scattering particle size analyzer (Otsuka, Electronics, DLS-700; the same applies hereinafter) was 30.2 nm. Transmission electron microscopic observation revealed that these fat emulsion particles were uniform spherical nanospheres and the lipid bilayer structure like a liposome was not observed.

Example 2

[0038] To 2 mg of amphotericin B (antifungal agent) were added 500 mg of soybean lecithin, 300 mg of cholesteryl oleate and 10 mL of distilled water for injection, and using a probe type ultrasonic homogenizer, the mixture was sorticated under ice-water cooling for 60 minutes. The amphotericin B-containing fat emulsion thus obtained was yellow and transparent. The emulsion was sterilized by filtration through a 0.22 μm membrane filter and filled in injection ampules, 2.0 mL per ampule, under nitrogen gas in a clean bench to give the inhalant of the invention. The average particle diameter of this inhalant fat emulsion as measured with a light scattering particle size analyzer was 40.2 nm. Transmission electron microscopic observation revealed that these fat emulsion particles were uniform spherical nanospheres and the lipid bilayer structure like a liposome was not observed.

Example 3

[0039] To 100 mg of guaiazulene (antiinflammatory agent) were added 400 mg of egg yolk lecithin, 270 mg of triolein and 10 ml of saline, and using a probe type ultrasonic homogenizer, the mixture was sonicated under ice-water cooling for 40 minutes. The guaiazulene-containing fat emulsion thus obtained was blue and transparent. The emulsion was sterilized by filtration through a 0.22 μm membrane filter and filled in injection ampules, 2.0 mL per ampule, under nitrogen gas in a clean bench to give the inhalant of the invention. The average particle diameter of this inhalant fat emulsion as measured with a light scattering particle size analyzer was 22.1 nm. Transmission electron microscopic observation revealed that these fat emulsion particles were uniform spherical nanospheres and the lipid bilayer structure like a liposome was not observed.

Example 4

[0040] To 1 mg of beclomethasone propionate (a steroid) were added 400 mg of egg yolk lecithin, 270 mg of medium-chain fatty acid triglyceride and 10 mL of distilled water for injection, and using a probe-type ultrasonic homogenizer, the mixture was sonicated under ice-water cooling for 50 minutes. The beclomethasone propionate-containing fat emulsion thus obtained was light yellow and transparent. The emulsion was sterilized by filtration through a 0.22 μm membrane filter and filled in injection ampules, 2.0 mL per ampule, under nitrogen gas in a clean bench to give the inhalant of the invention. The average particle diameter of this inhalant fat emulsion as measured with a light scattering particle size analyzer was 35.2 nm. Transmission electron microscopic observation revealed that these fat emulsion particles were uniform spherical nano spheres and the lipid bilayer structure like a lipogome was not observed.

Example 5

[0041] To 50 mg of cyclosporin A (immunosuppressant) was added 500 mg of purified egg yolk lecithin, 500 mg of purified soybean oil, 9 mL of distilled water for injection, and further 220 mg of glycerin JP, and using a probe-type ultrasonic homogenizer, the mixture was sonicated under ice-water cooling for 60 minutes. The cyclosporin A-containing fat emulsion thus obtained was light yellow and transparent. This emulsion was diluted with distilled water for injection to make 10 mL and filtered through a 0.22 μm membrane filter and the sterile filtrate was filled into injection ampules, 2.0

Example 11

[0047] To 0.2 g of tulobuterol (β2 adrenergic agonist) were added 50 g of egg yolk lecithin, 50 g of rapaseed oil and 1 L of 10% sucrose, and the mixture was emulsified with a microfluidizer type homogenizer (M110-E/H). The tulobuterol-containing fat emulsion thus obtained was off-white and transparent. The average particle diameter of this fat emulsion as determined with a light scattering particle diameter analyzer was 36.6 nm. This emulsion was sterilized by filtration through a 0.22 μm membrane filter and filled in injection vials, 2.0 mL per vial, in a clean bench, followed by freeze-drying to give a lyophilized version of the inhalant of the invention. This lyophilized inhalant was reconstituted with distilled water for injection and the average particle diameter of the fat emulsion was determined with a light scattering particle size analyzer. The result was 38.7 nm. Transmission electron microscopic observation revealed that this fat emulsion comprised uniform spherical nanospheres and no lipid bilayer structure like a liposome was observed.

Example 12

[0048] The lyophilized inhalant of the invention (250 g) as obtained in Example 11 was micronized to a particle diameter of 0.5~4 μm and filled in hard capsule shells, 0.5 g per capsule. By this procedure, 1000 capsules each containing 0.5mg of tulobuterol were obtained. The capsule was pierceable with a pulverizer-powder inhaler (JP Koho S63-6024) whereby the contents were made inhalable.

Test Example 1

Determination of mass median aerodynamic diameter (MMAD) and its distribution (I)

[0049] The CA-containing inhalant of the invention as prepared in Example 1 was used as a test sample and a known fat emulsion having an average particle diameter of 0.2 μm in which CA had been entrapped was used as a control sample. This control sample was prepared by adding 9 mL of distilled water for injection to a mixture of 5 mg of CA, 100 mg of purified soybean oil and 12 mg of purified egg yolk lecithin, further adding 220 mg of glycerin JP, homogenizing the whole mixture with a probe type ultrasonic homogenizer under ice-water cooling, and making up the emulsion to 10 mL with distilled water for injection.

[0050] The measurement of mass median aerodynamic diameter and its distribution was carried out with Anderson's Cascade Impactor (listed in USP) which classifies particles into multiple stages by utilizing differences in inertia in the aspiration of an aerosol at a constant speed.

[0051] In the experiment, a nebulizer body (Medical Device Approval No. (55B) 1329; the same applies hereinafter] was attached to a Nissho model compressor [Medical Device Approval No. (55B) 1270; the same applies hereinafter] in the first place and each sample was sprayed at a flow rate of 6 L/min. for 80 minutes to generate a mist of aerosol particles. The aerosol particles thus produced were aspirated with a vacuum pump at a flow rate of 28.3 L/min. and classified into multiple stages. The aerosol particles captured in each stage were washed with methanol and recovered, and its fluorescence intensity was measured to estimate the amount of the drug. The results are shown in Fig. 1.

[0052] It can be seen from Fig. 1 that, compared with the control sample, the test sample gave larger drug amounts in the stages from 0 to 2.1 μm, with a significant difference at $p<0.01$. In particular, whereas the control sample was scarcely captured in the stages up to 2.1 μm, about 70% of the total amount of the drug recovered was found in these stages. This is probably because particles of small mass median aerodynamic diameter could be produced by reducing the particle diameter of the fat emulsion. In the stages >2.1 μm ~ ≤9 μm, no significant difference was found at the $p<0.05$ level between the two groups. It was also confirmed that the total amount of the drug recovered in all the stages was about 3-fold greater in favor of the test sample as compared with the control sample.

[0053] The mass median aerodynamic diameter is a factor of great importance for the drug to reach and get deposited at the target site. In humans, the mass median aerodynamic diameter of particles entering the airway is considered to be 1~10 μm and it is acknowledged that aerosol particles within the diameter range of 2~5 μm, in particular, are optimal for the drug reaching and getting deposited in the airway (the bronchus to the terminal bronchiole) and that the particles capable of reaching the alveolus located deeper is 1~2 μm in diameter (JP Forum Vol. 4, No. 1, 1995). As can be readily inferred from the results of this Test Example 1 in which the test sample was found to be significantly rich in the fraction of aerosol particles not greater than 2.1 μm in diameter as compared with the control sample, the inhalant of the invention easily generates aerosol particles 1~2 μm in diameter which can hardly be obtained with the conventional fat emulsion. Thus, it can be suggested that the delivery of the drug deep into the alveolus which could not be achieved with the conventional fat emulsion can now be easily accomplished in accordance with present invention.

Table 1

	Filtrate (mL)	Drug recovery (%)
Test sample	9.9±0.2	100±1.3
Control sample	1.3±0.1	12±2.3

[0061] It will be apparent from Table 1 that whereas the control sample could hardly be filtration-sterilized, the test sample could be effectively filtration-sterilized.

Test Example 5

Transpulmonary administration experiment in rabbits (-1)

[0062] Using 6 male 9-week-old rabbits (Kbs: JW), the trachea was exposed under anesthesia and connected to a Y-cannula and the animal was placed on supportive respiration using a respirator. Under supportive respiration, the inhalant according to Example 5 of the present invention as a test sample and the same 0.2 μm fat emulsion as used in test Example 1 in which cyclosporin A had been entrapped as a control sample were administered each in a dose of 5 mg/kg by 30 (ca) minutes' spraying using a Nissho model compressor and a nebulizer body connected thereto. After completion of inhalation, the cannula was disconnected and the cannulation wound was sutured. Then, the blood was drawn from the auricular vein at timed intervals and the time course of plasma cyclosporin A concentration was monitored by fluorescence polarization immunoassay (FPIA). The results are shown in Fig. 4.

[0063] It will be apparent from Fig. 4 that the plasma cyclosporin A concentration was consistently higher in the test sample administration group than in the control sample administration group, with a difference of about 3-fold in the area under the plasma concentration-time curve (AUC). Thus, although the translocation of an inhaled drug into the systemic circulation depends upon arrival of the drug at the alveolus, the control 0.2 μm (dia.) fat emulsion is hardly able to deliver the drug to the alveolus. In the case of the inhalant of the invention, as can be seen if only from the result of Test Example 1, the drug is entrapped in aerosol vesicles capable of reaching the alveolus. It is obvious from the result of this transpulmonary administration experiment in rabbits that the inhalant of the invention as the test sample is outstanding in the ability to reach the alveolus.

Test Example 6

Transpulmonary administration experiment in rabbits (-2)

[0064] Using 6 male 9-week-old rabbits (Kbs: JW), the trachea was exposed under anesthesia and connected to a Y-cannula and the animal was placed on supportive respiration using a respirator. Under supportive respiration, the inhalant according to Example 5 of the invention as a test sample and an inhalant comprising cyclosporin A solubilized with Tween-80 as a control sample were administered each in a dose of 1 mg/kg by 30 (ca) minutes' spraying using a Nissho model compressor and a nebulizer body attached thereto. After completion of inhalation, the cannula was disconnected and the cannulation wound was sutured. Then, the blood was drawn from the auricular vein at timed intervals and the time course of plasma cyclosporin A concentration was monitored by fluorescence polarization immunoassay (FPIA). The results are shown in Fig. 5.

[0065] It can be seen from Fig. 5 that the AUC showed no difference at the $p<0.05$ level between the test sample administration group and the control sample administration group. The two groups were almost comparable in the time course of plasma concentration up to 2 hours but the concentration in the test sample administration group after 3 hours declined slightly as compared with the control sample administration group. Thus, the inhalant of the invention as the test sample was superior to the surfactant-solubilized control inhalant in the slow and prolonged release characteristics in the plasma.

Test Example 7

Influence of the solubilization-effective surfactant concentration on sprayability

[0066] The samples of CA, guaiazulene and dexamethasone palmitate each solubilized with HCO-60, propylene glycol, sodium lauryl sulfate, Tween-80 or Triton X100 were compared with the inhalants according to Examples 1, 3

BRIEF DESCRIPTION OF THE DRAWINGS

[0072]

Fig. 1 represents the amount of the drug in each aerosol particle size stage. The abscissa represents the impactor stage size ranges (μm) and the ordinate represents the fluorescent intensity. The solid bar represents the inhalant of the invention and the open bar represents the control.

Fig. 2 shows the amount of the drug in each aerosol particle size stage. The abscissa represents the impactor stage size ranges (μm) and the ordinate represents the fluorescent intensity. The solid bar represents condition-1 and the open bar represents condition-2.

Fig. 3 shows the time course of concentration of the spray solution. The abscissa represents time (min.) and the ordinate represents the fluorescent intensity. -●- represents the test sample (the inhalant of the invention); -○- represents control sample-1; and -△- represents control sample-2 (physiological saline).

Fig. 4 shows the results of a transpulmonary administration experiment in rabbits. The abscissa represents time (hr.) and the ordinate represents the plasma concentration of cyclosporin A (ng/ml). -●- represents the inhalant of the invention and -○-represents the control inhalant.

Fig. 5 shows the results of a transpulmonary administration experiment in rabbits. The abscissa represents time (hr.) and the ordinate represents the plasma concentration of cyclosporin A (ng/ml). -●- represents the inhalant of the invention and -○-represents the control inhalant.

Claims

1. A fat emulsion for inhalational administration, or a lyophilized composition thereof, which is an o/w fat emulsion comprising fat emulsion particles essentially composed of an oil component, an emulsifying agent and a drug as dispersed in water, characterized in that the average particle diameter of said fat emulsion particles lies within the range of 5~100 nm.

2. The fat emulsion or lyophilized composition thereof as claimed in Claim 1 wherein the proportion of said oil component of the fat emulsion lies within the range of 0.1~30 w/v % and the proportion of said emulsifying agent lies within the range of 0.05~40 w/v %.

3. The fat emulsion or lyophilized composition thereof as claimed in Claim 1 or 2 wherein the weight ratio of said oil component to said emulsifying agent (oil/emulsifier ratio) lies within the range of 0.1~20.

4. The fat emulsion or lyophilized composition thereof as claimed to any of Claims 1~3 wherein the oil component is a vegetable oil or a glyceride and the emulsifying agent is a phospholipid or a nonionic surfactant.

5. The fat emulsion or lyophilized composition thereof as claimed in Claim 4 wherein the vegetable oil is soybean oil and the phospholipid is egg yolk lecithin.

6. The fat emulsion or lyophilized composition thereof as claimed in any of Claims 1~5 further comprising a saccharide.

7. A lyophilized composition obtainable by freeze-drying the inhalant fat emulsion of Claim 6 wherein the amount of said saccharide in the fat emulsion is 1~30 w/v %.

8. A lyophilized composition of the inhalant fat emulsion of Claim 6 or 7 wherein the saccharide is a disaccharide.

9. The inhalant fat emulsion or lyophilized composition thereof as claimed in any of Claims 1~8 further comprising a fatty acid and/or cholesterol.

10. A nebulizer preparation comprising the inhalant fat emulsion or lyophilized composition thereof claimed in any of Claims 1~9.

11. A powdery inhalant comprising a lyophilized composition of the inhalant fat emulsion claimed in any of Claims 1~9.

12. A method of administering a fat emulsion or a lyophilized composition thereof by way of inhalation, said fat emulsion being an o/w fat emulsion comprising fat emulsion particles essentially composed of an oil component, an emulsi-

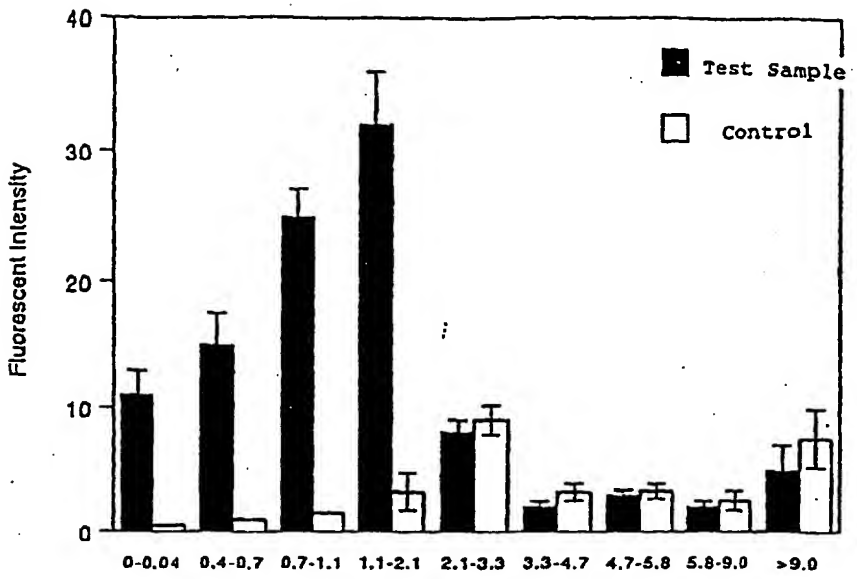
40
30
20
10
0
Fluorescent Intensity
Test Sample
Control
0-0.04
0.4-0.7
0.7-1.1
1.1-2.1
2.1-3.3
3.3-4.7
4.7-5.8
5.8-9.0
>9.0
Impactor stage size ranges (μm)

Fig.1

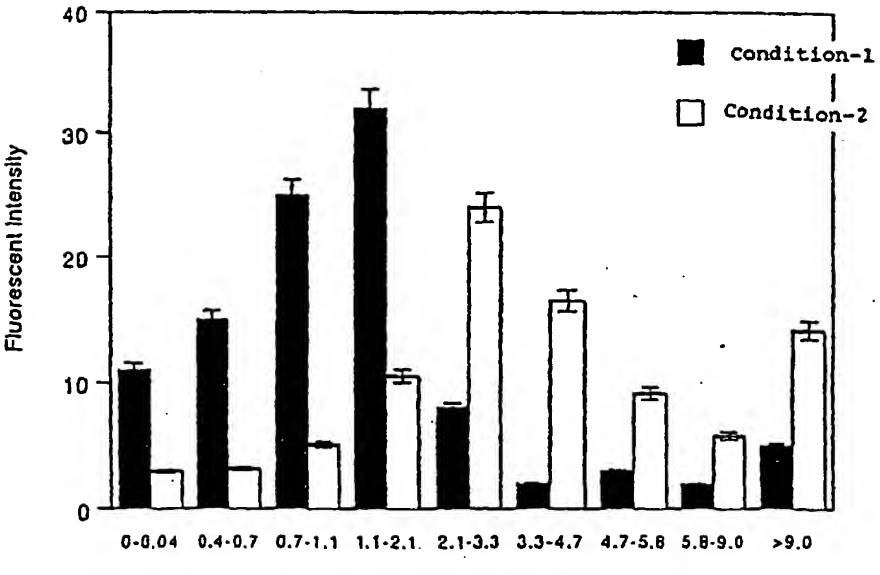
40
30
20
10
0
Fluorescent Intensity
Condition-1
Condition-2
0-0.04
0.4-0.7
0.7-1.1
1.1-2.1
2.1-3.3
3.3-4.7
4.7-5.8
5.8-9.0
>9.0
Impactor stage size ranges (μm)

Fig.2

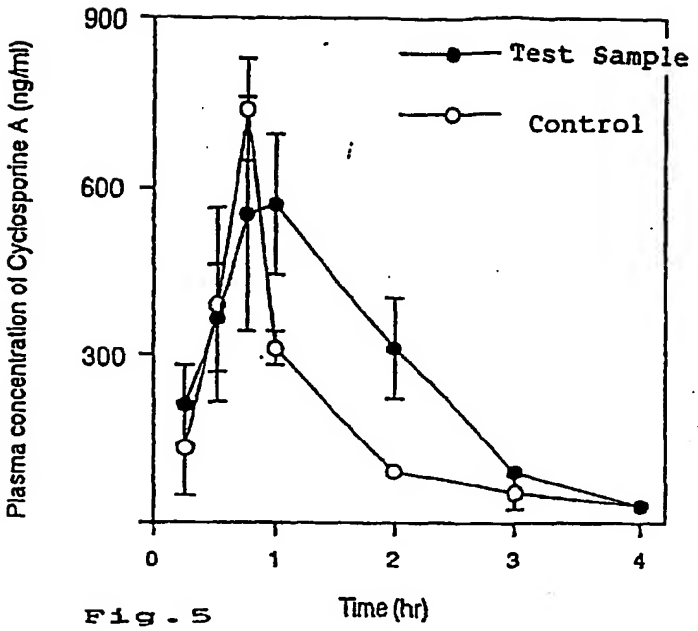
900
600
300
0
1
2
3
4
Plasma concentration of Cyclosporine A (ng/ml)
Time (hr)
Test Sample
Control

Fig. 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP99/01004

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [x] Claims Nos.: 12

because they relate to subject matter not required to be searched by this Authority, namely:

Claim 12 involves methods for treatment of the human body by therapy and thus relates to a subject matter which this International Searching Authority is not required, under the provisions of Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.

2. [ ] Claims Nos.:

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1992)